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UV Spectrophotometric Method for the Estimation of Roflumilast in Human Serum

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Abstract

Ultra violet spectrophotometric method for the estimation roflumilast in spiked human serium. The present study was spectrophotometric estimation of roflumilast in spiked human serum is based on measurement at the maximum wavelength 248 nm using 0.2M Hcl as a solvent. The roflumilast stock solution was prepared with distilled water to produse calibration curve. The standard solution of roflumilast shows absorption maxima at 248 nm. The roflumilast obeyed beer lamberts law in the concentration range of 40-88µg/ml with regression 0.9987 at 248nm. The overall percentage recovery of roflumilast was found to be 99.52% which reflects that the method was free from the interference. The % RSD values of intra day and inter day precision was found to be 0.031 and 0. 046% respectively, which is less than 2 and hence this method is precise. The values of LOD and LOQ were found to be 0.783 and 2.375 µg/ml, respectively and it indicates this method was sensitive.

Keywords: Serum; Roflumilast; Method development; Spectrophotometric

Introduction

Roflumilast (Figure 1) is a chemically3-(cyclopropylmethoxy)-N-(3,5-dichloropyridin-4-yl)-4-(difluoromethoxy) benzamide and it is a selective, long acting phosphodiesterase-4 inhibitor.

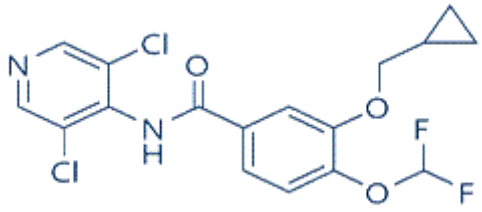

Figure 1: Chemical structure of roflumilast.

Roflumilast was used for the treatment of chronic obstractive pulmonary disease [1,2] and roflumilast was freely soluble in methanol and lower alcohols. A few spectrophotometric [3-5] and HPLC [6,7] methods were reported earlier for the estimation of roflumilast in bulk and pharmaceutical dosage forms and this method was validated as per ICH guidelines [8]. Although no method was reported for the estimation of roflumilast in serum in pharmaceutical dosage forms. The present study was authors reported a simple, sensitive, accurate and precise for estimation of roflumilast in spiked human serum by using UV spectroscopy.

Experimental

Instrument

ElicoSL164 UV-Visible spectrophotometer with double beam detector configuration. The above instruments had automatic wavelength accuracy 0.1nm and matched quartz cells and Weighing balance (Elico, India).

Materials

Blank human serum, Roflumilast sample (99.90% purity) was purchased from Spectrum Pharma Research Solutions, Hyderabad, India. Hydrochloiric acid and water (HPLC grade) obtained from Qualigens Fine Chemicals, Mumbai, India.

Method development

Preparation of standard solution: A standard stock solution was prepared by accurately weighed 1000 mg of roflumilast in 1000ml of volumetric flask and dissolved in 0.2M of Hcl to obtain a concentration 1000 µg/ml. Further diluting 2.5ml of stock solution to 25ml with distilled water to get desired concentration of 25µg/ml. Further diluting 0.4ml of stock solution to 100ml with distilled water to get desired concentration of 40µg/ml.

Selection of wavelength for analysis of Roflumilast: Accurately measured 0.4ml of standard stock-III was transferred in to 100ml volumetric flask and diluted to 100ml to give concentration of 40µg/ml and it was used for initial spectral scan in the range of 200-400 nm to detect maximum wavelength and further dilutions for linearity was

prepared from the stock solution by allegation method. The maximum wave length was found at 248nm.

Preparation of calibration plot: 5ml of serum was taken in a 250 ml separating funnel and spiked with 10ml aqueous solution contains 40-88 µg/ml of Roflumilast (40, 50, 64, 72, 88 µg/ml).

To the same solution add 20 ml of methyl acetate. The content was shaken for 15 min and the liquids were allowed to separate in to two immiscible phases.

The lower aqueous layer was discarded and the upper organic layer was collected in a beaker. Finally water free organic layer was transferred in to a dried beaker and evaporated to dryness on a hot water bath. The dry residue was reconstituted with 2ml of 0.2M HCL and transferred in to a 25ml calibrated flask. The volume was making with water.

Method validation

The developed method was validated for various parameters like linearity, precision, accuracy, limit of detection, limit of quantitation, robustness and ruggedness according to ICH guidelines.

Linearity: The manifest linear relationship in the range of 40-88 µg/ml of roflumilast.

Precision: The intra day precision analysed in the same day and inter day precision analysed for three consecutive days. The results was indicated by calculated percent relative standard deviation.

Accuracy: Recovery study was carried out at three different levels 50%, 100% and 150%. The percentage recovery was calculated as mean ± standard deviation.

Limit of detection (LOD): Formula for measuring of limit of detection.

LOD = 3.3 σ/S

Where,

σ = Standard deviation

S = Slope

Limit of quantification (LOQ): Formula for measuring of limit of quantification.

LOD = 10 σ/S

Where,

σ = Standard deviation

S = Slope

Robustness: The robustness of an analytical procedure is a measure of its capacity unaffected by a small.

Ruggedness: The ruggedness to express in condition with variations like different analyst, different instruments and different days.

Results and Discussion

Selection of wavelength

The spectra of roflumilast in 0.2M hydrochloric acid showed absorption at 248 nm shown in Figure 2.

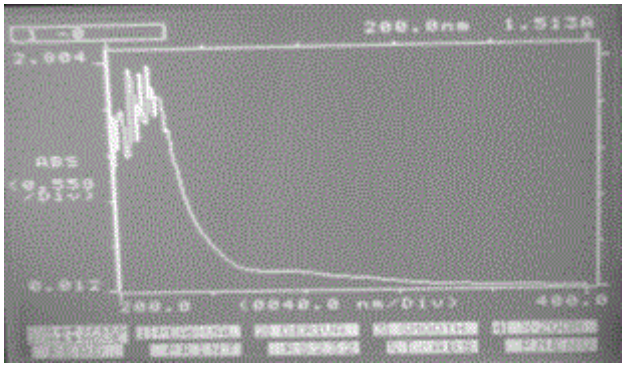

Figure 2: UV spectrum of roflumilast.

Linearity

The linearity for the proposed method was investigated at five concentration levels (40-88 µg/ml) of reference standard roflumilast. The linearity was shown in Table 1 and Figure 3.

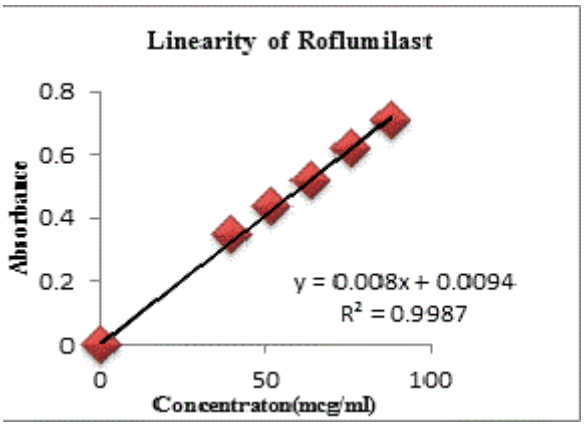

Figure 3: Linearity plot of roflumilast.

S.No.	Concentration (mcg/ml)	Absorbance
1	40	0.345
2	52	0.472
3	64	0.521
4	72	0.643
5	88	0.712

Table 1: Linearity data of roflumilast.

Precision

The percent RSD value of intra day and inter day precision were found to be 0.031 and 0.046 respectively, as shown in Table 2.

Sample number	Assay of roflumilast	Assay of roflumilast

	Intra-day precision	Inter-day precision
1	99.13	99.13
2	99.11	99.18
3	99.09	99.08
4	99.07	99.08
5	99.05	99.10
6	99.12	99.05
Mean	99.09	99.10
%RSD	0.031	0.046

Table 2: Precision data of roflumilast.

Accuracy

The percentage of recovery of roflumilast was found to be 99.82 %, shown in Table 3.

Ingredient	Tablet amount (mg/ml)	Level of Addition (%)	Amount added (mg)	Drug found (mg/ml)	% Recovery	Average % recovery
Roflumilast	500	50	5	4.97	99.40	
	500	100	10	9.99	99.90	99.52 ± 0.13
	500	150	15	14.89	99.26	

Table 3: Accuracy data of roflumilast.

Limit of detection

The limit of Detection (LOD) value obtained was 0.783 µg/ml, it indicates the high sensitivity of the proposed method Table 4.

S.No	Parameter	Roflumilast
1	Absorption maxima(nm)	248
2	Linearity (mcg/ml)	40-88
3	Standard Regression Equation	Y = 0.008X - 0.0094
4	Correlation Coefficient (r2)	0.9987
5	Molar extinction coefficient	0.008
6	Accuracy (%recovery ± SD)	99.52 ± 0.13
7	Precision	99.09% (Intra-day precision) and 99.10% (Inter-day precision)
8	Sandell's Sensitivity (mg/cm2/0.001absorbance unit)8	0.155
9	LOD (mg/ml)	0.78
10	LOQ (mg/ml)	2.37

Table 4: Validation parameters of roflumilast.

Limit of quantitation

The limit of quantitation (LOQ) obtained was 2.375 µg/ml, it indicates the high sensitivity of the proposed method.

Robustness

The percent RSD of roflumilast was found to be 0.966. The robustness of results were shown in Table 5.

S.No.	Roflumilast
Assay-1	99.13
Assay-2	99.17
Assay-3	99.16
Assay-4	99.12
Assay-5	99.19
Assay-6	99.2
Mean	99.17
S.D	0.09
%RSD	0.091

Table 5: Robustness data of roflumilast.

Ruggedness

Percent relative standard deviation of roflumilast was found to be 0.719. The ruggedness of results were shown in Table 6.

S.No	Roflumilast
Assay-1	99.13
Assay-2	99.18
Assay-3	99.19
Assay-4	99.16
Assay-5	99.09
Assay-6	99.15
Mean	99.15
S.D	0.036
%RSD	0.033

Table 6: Ruggedness data of Roflumilat.

Conclusion

The proposed method was simple, linear, precise, accurate and robust developed for the estimation of roflumilast in human serum by using UV spectroscopy. The method was validated as per the ICH guidelines. Hence, The present method can be adopted for routine in quality control and reaserch industries.

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